AD NUMBER
AD811760
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative and Operational Use; Mar 1967. Other requests shall be referred to the Army Biological Research and Development Laboratory, Fort Detrick, MD 21701.
AUTHORITY
BDRL, per d/a ltr, 28 Sep 1971

AD

AD811760

TECHNICAL MANUSCRIPT 350

PATHOPHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN ANTHRAX

Jerry S. Walker
Ralph E. Lincoln
Frederick Klein

MARCH 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 350

PATHOPHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN ANTHRAX

Jerry S. Walker

Ralph E. Lincoln

Frederick Klein

Process Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORY

Project 1C522301A059

March 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

PATHOPHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN ANTHRAX

ABSTRACT

Pathophysiological and biochemical changes in anthrax are reviewed. The comparison between spore- and toxin-challenged animals shows a remarkable similarity, indicating that the toxin of _Bacillus anthracis_ caused death although the organisms per se caused little pathophysiological response. Death was primarily due to a respiratory depression of central nervous system origin; the cardiovascular system remained intact. Death occurred with an extreme anoxi· that was accompanied late in the disease by numerous secondary or nonspecific changes in the blood cellular, chemical, and gaseous elements.

Anthrax is a disease in which, as repeatedly mentioned in the literature, the signs or symptoms are not consistent with the severity of the disease and imminence of death.

Lack of marked specific pathophysiological changes may well be the reason why no extensive physiological study was made during the era dominated by the histopathologist.

In 1955, Smith et al.[1] demonstrated that _Bacillus anthracis_ produced toxin in vivo; they later showed that toxin could be produced in vitro. As work in this area increased, better tests for toxin were developed and, coincidentally, equipment was transistorized and developed as a routine tool for physiological monitoring of host responses.

In 1960 and 1961, when our group started its research on anthrax pathogenesis, there were several hypotheses for the cause of death: (i) capillary blockage by bacilli,[2] (ii) kidney shutdown and progressive secondary shock,[1] (iii) attachment of toxin to white blood cells (WBC) and destruction of reticuloendothelial system (RES) cells,[3] and (iv) altered capillary permeability and hypotension, which perhaps is the basis of the more recently proposed causes, pulmonary edema.[4] We have subsequently suggested central nervous system (CNS) depression and respiratory failure[5] as the cause of death.

We will review the pathophysiological data from the standpoint of (i) changes in blood cellular elements, (ii) changes in blood gas and chemical constituents, (iii) other signs and symptoms, and (iv) other physiological changes. We shall, when possible, compare the infectious disease with that caused by the sterile toxin of _B. anthracis_.

A review of the literature showed that, although a number of biochemical parameters have been investigated, it still was impossible to identify the system or systems affected that resulted in the death of the host. There was general agreement that WBC count increased and that pO_2 decreased markedly. There was little to indicate that the disease caused by the organism was different from that by the toxin. We measured a number of physiological parameters in spore- and toxin-challenged rhesus monkeys, chimpanzees, and rats to find if there were a similarity or response among species and if toxin alone would produce changes similar to those observed during infection and, hopefully, to give us a clue to the system or systems primarily affected.

Five rhesus monkeys and four chimpanzees were challenged by either the aerosol or intradermal route with spores, and the course of the disease was characterized.[6] Each animal served as his own control in that he was monitored several hours before challenge to establish base line values. The blood cellular responses (Fig. 1 and 2) are shown as statistically fitted lines. Increases in WBC counts and hematocrit values occurred very late in the course of disease. Blood glucose and calcium were decreased, but total protein remained within normal limits (Fig. 3). During septicemia some monkeys developed a precipitous fall in blood glucose and died with terminal blood glucose levels of approximately 30 mg per 100 ml. The blood chemical parameters assayed in the chimpanzees were glucose and the electrolytes Na^+, K^+, and Cl^- (Fig. 4). The precipitous fall in glucose level was again seen, with some animals showing levels of only 10 mg per 100 ml terminally. Na^+ also decreased; K^+ and Cl^- increased terminally. The increased K^+ is partly attributable to lysis of erythrocytes and somatic cells. Blood pH and gas changes monitored on the same animals indicated that two systems were involved (Table 1). A respiratory alkalosis occurred in early septicemia; then, terminally, a metabolic acidosis was superimposed.

The responses of seven rhesus monkeys challenged intravenously with toxin (Fig. 5 and 6) were essentially identical with those observed for the spore-challenged animals; however, the changes occurred relatively earlier in the course of disease. Phosphorus showed a slight increase in concentration.

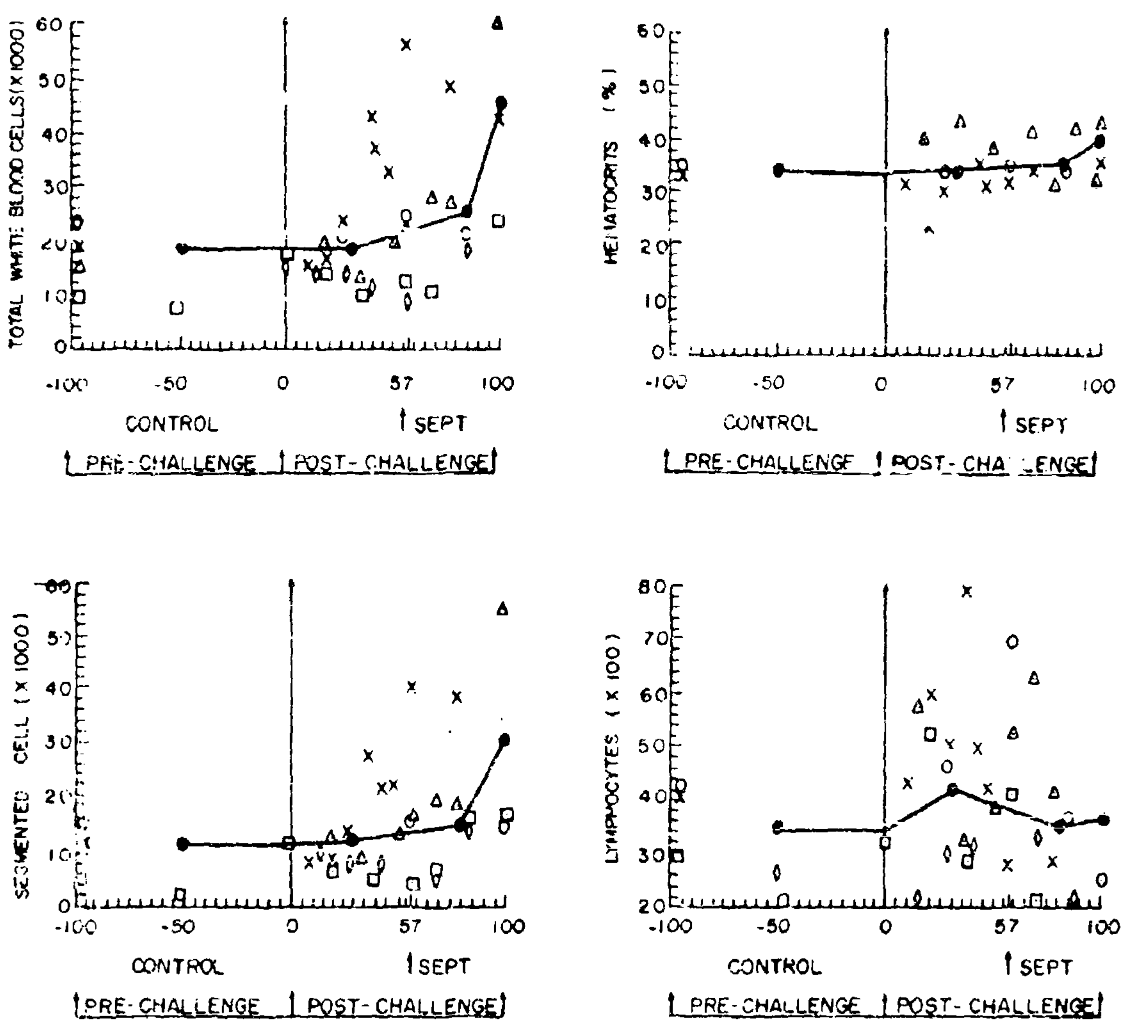

Figure 1. Individual and Mean Blood Cellular Values of Five Monkeys Following Aerosol or Intradermal Challenge with the Vlb Strain of B. anthracis. Relative time is a mathematical expression of actual responses to allow presentation of the data.[6]

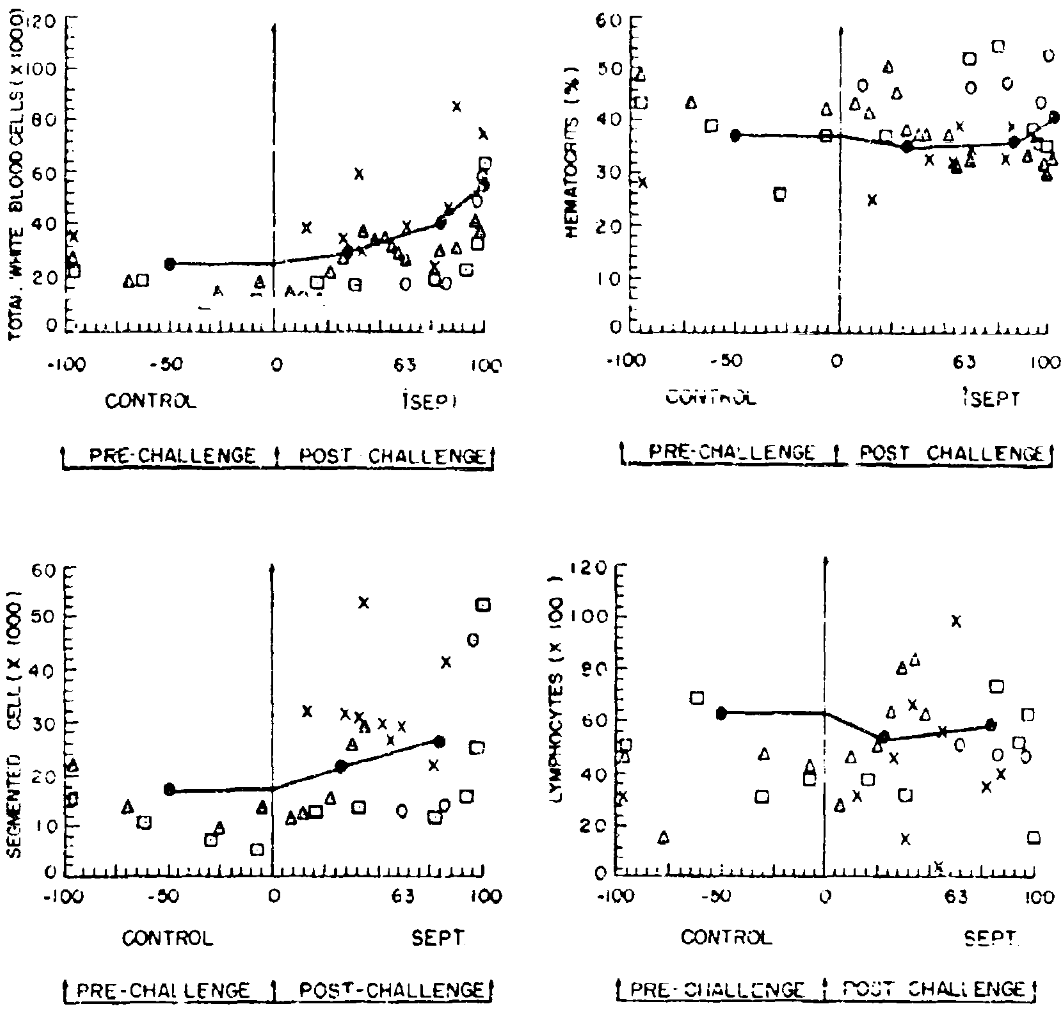

Figure 2. Individual and Mean Blood Cellular Values of Four Chimpanzees Following Aerosol Challenge with the V1b Strain of *B. anthracis*. Relative time is a mathematical expression of actual responses to allow presentation of the data.

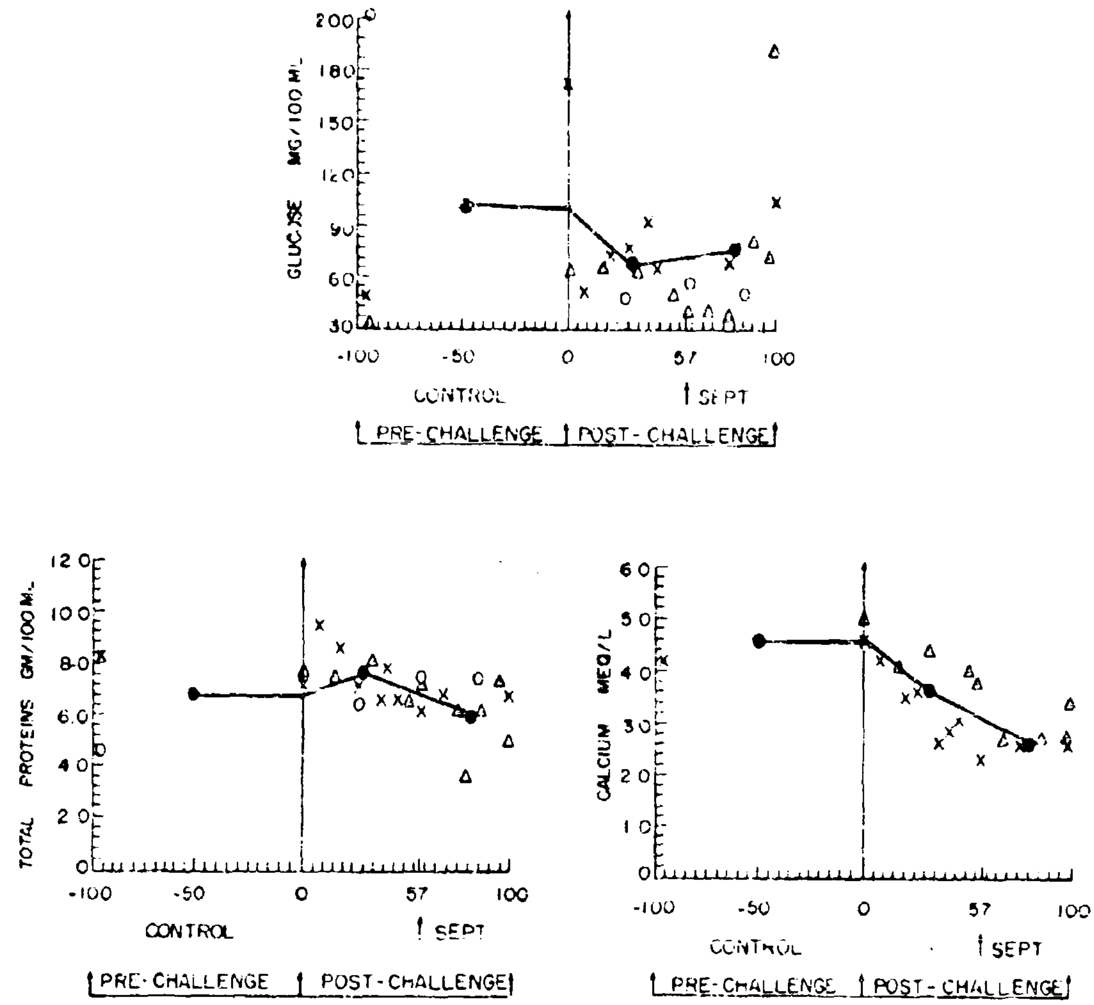

Figure 3. Individual and Mean Blood Chemical Values of Five Monkeys Following Aerosol or Intradermal Challenge with the V1b Strain of *B. anthracis*. Relative time is a mathematical expression of actual responses to allow presentation of the data.[e]

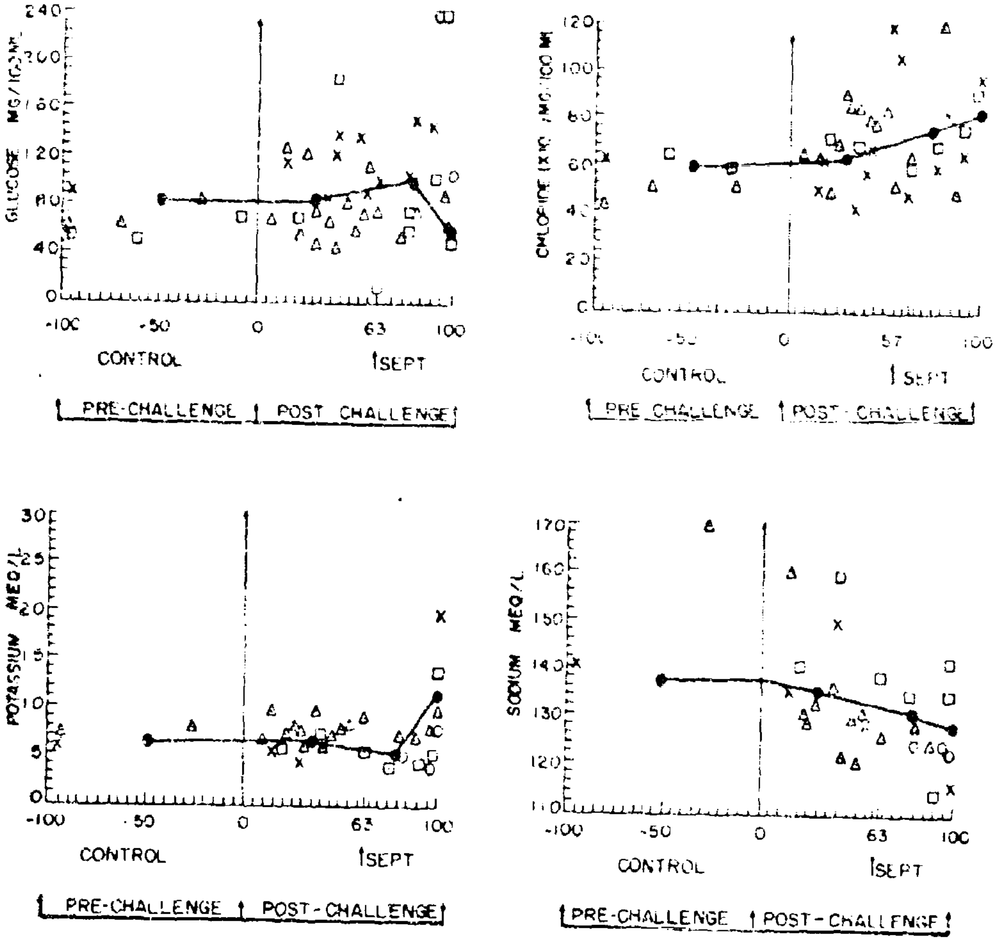

Figure 4. Individual and Mean Blood Chemical Values of Four Chimpanzees Following Aerosol Challenge with the V1B Strain of *B. anthracis*. Relative time is a mathematical expression of actual responses to allow presentation of the data.

TABLE 1. BLOOD pH AND GASES IN UNINFECTED AND INFECTED CHIMPANZEES BEFORE AND AFTER AEROSOL EXPOSURE TO TO V1b STRAIN OF B. ANTHRACIS[a]

Blood Characteristic	Pre-Challenge	Post-Challenge	Septicemia
pO_2	47.5	47.7	27.5
pCO_2	47.2	43.2	39.8
pH	7.39	7.49	7.45

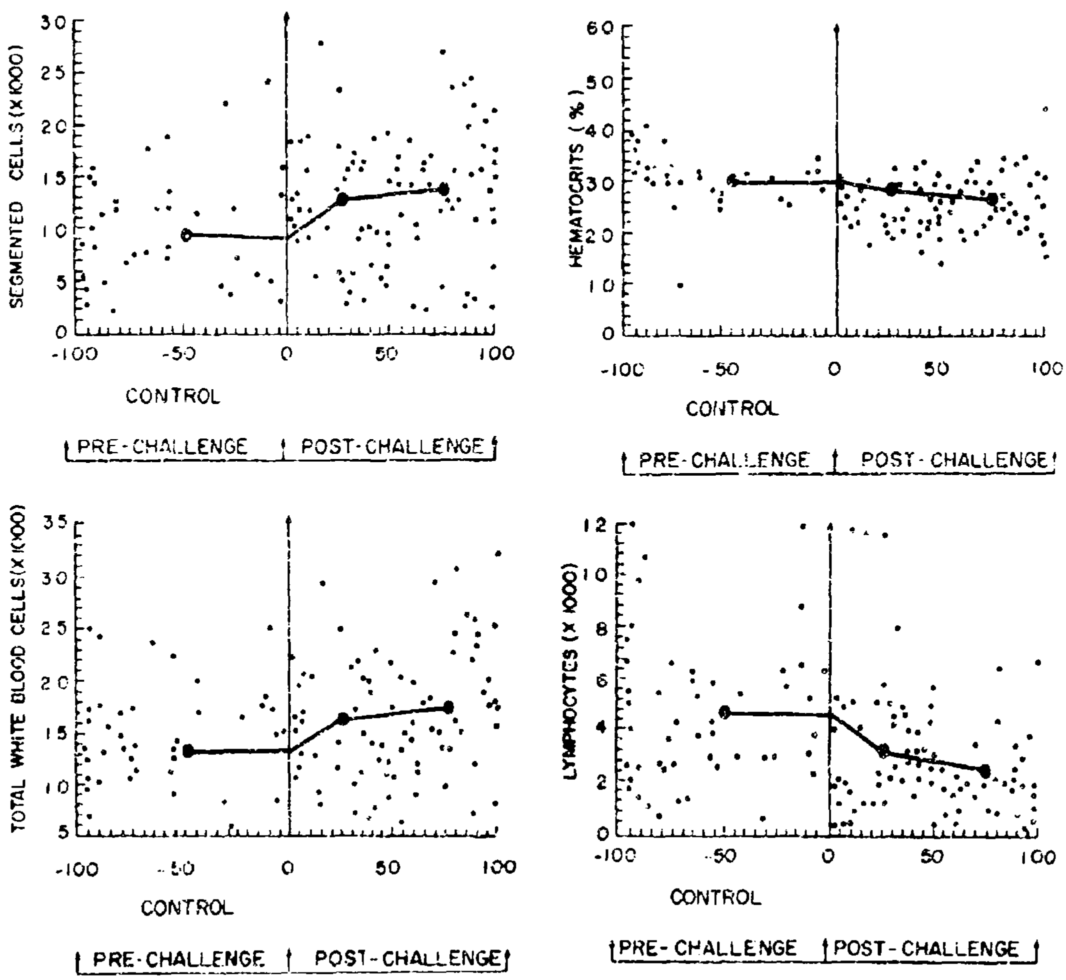

Figure 5. Individual and Mean Blood Cellular Values of Seven Monkeys Following Intravenous Challenge with B. anthracis Toxins. Relative time is a mathematical expression of actual responses to allow presentation of the data.[a]

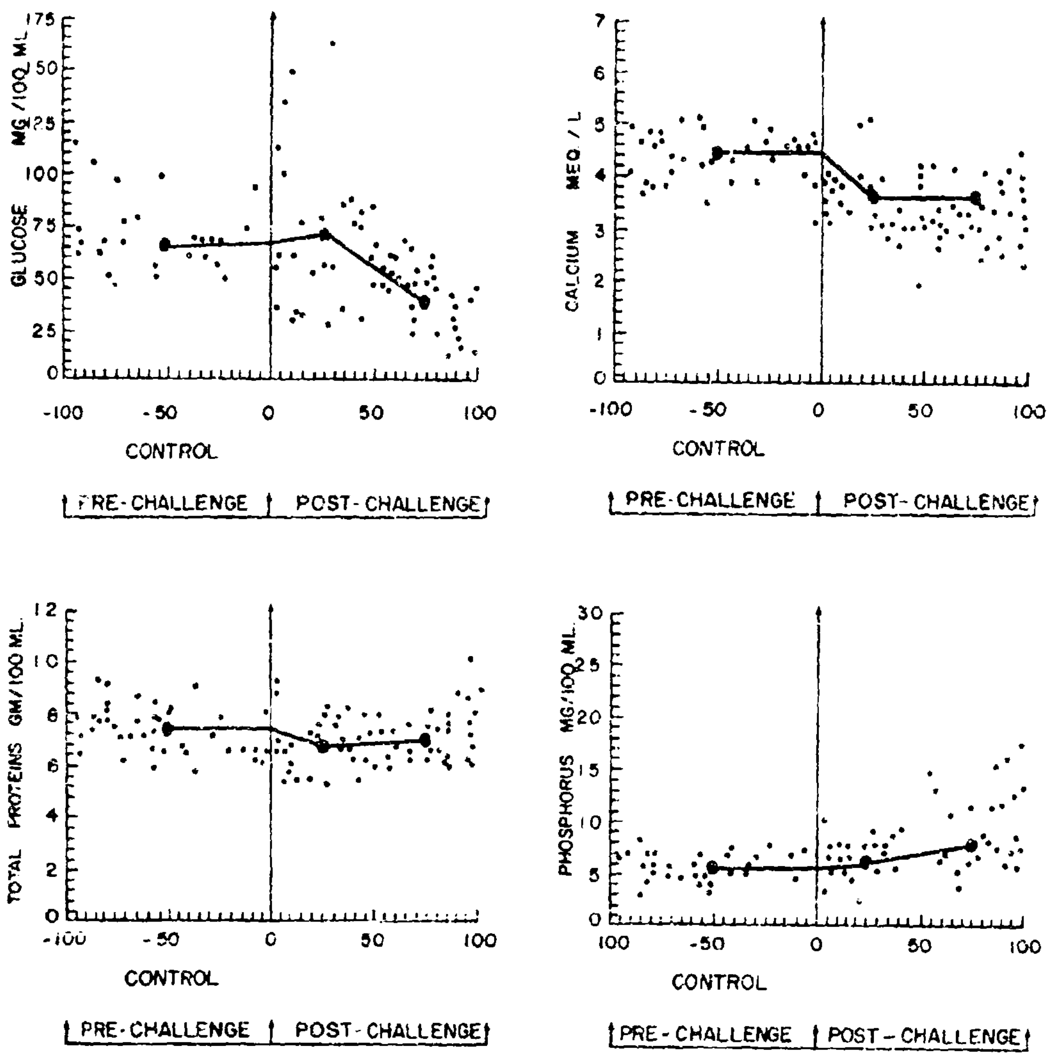

Figure 6. Individual and Mean Blood Chemical Values of Seven Monkeys Following Intravenous Challenge with B. anthracis Toxins. Relative time is a mathematical expression of actual responses to allow presentation of the data.[F]

The decreased calcium concentration probably is the result of the lowered pH because the ratio between protein-bound calcium and free calcium is pH-dependent. The changes observed in calcium and potassium levels allow us to explain the hyperesthesia and muscle spasms seen in some humans and animals dying of anthrax as neuromuscular irritability that is enhanced by either a decrease in calcium or an increase in potassium. In anthrax, both changes occur. It is probable that more humans and animals do not display this clinical syndrome because of the extreme hypoxia and hypoglycemia that occur. Whether or not a quiet or a violent death occurs depends on which of the above systems is altered more at the time of death. The blood cellular responses in the rabbit were identical to those of the monkey and chimpanzee for both toxin and spore challenge. The dramatic changes in blood glucose levels we observed in the primates and others observed in rabbits, rats, and sheep led us to follow these changes in the Fischer 344 rat. When rats were challenged with whole toxin or its lethal factor (LF) and protective antigen (PA) components combined (Table 2), not only was serum glucose lowered to hypoglycemic levels, but a decrease in liver and muscle glycogen followed in that order. These extremely low glucose and glycogen levels observed in toxin-challenged rats also occurred in spore-challenged rats.

TABLE 2. GLUCOSE AND GLYCOGEN LEVELS IN FISCHER 344 RATS

Challenge	Time	Serum Glucose, mg %	Tissue Glycogen, mg % Liver	Leg Muscle
Lethal Preparations				
Spores	0 hr	68		
	16 hr	48		
	24 hr	23		
Whole Toxin	0 min	69	243[a]	
	80 min	96	205[a]	
	140 min	64	49[a]	
Protective Antigen	0 min	77	131	144
and	80 min	88	66	124
Lethal Factor	140 min	98	17	113
Non-Lethal Preparations				
Protective Antigen	0 min	73	131	144
	80 min	59	72	124
	140 min	82	41	121
Lethal Factor	0 min	73	131	144
	80 min	91	62	133
	140 min	73	38	132

a. Food had not been withheld from this group for 24 hours prior to challenge as it had been for all other groups. The data from this group were not included in the statistical analysis.

The monkey and chimpanzee were used for further physiological studies. Monkeys were challenged by either spores or toxin; the chimpanzees were challenged only with toxin. The parameters monitored were EEG, heart rate, EKG, respiration, phrenic nerve discharge, and blood pressure. Depression of the EEG and a decrease in the respiration rate occurred within 5 to 8 minutes after injection of 10,000 units of toxin in the rhesus monkey and 100,000 units of toxin in the chimpanzee. One-third of the animals became comatose but recovered rapidly. The EEG then remained essentially normal until a few hours before death, although a cyclic type of depression was observed in some animals. The response of one animal that is typical of the group is shown in Figure 7. At 26 hours post-challenge, respiration ceased. Death was preceded by 6 hours of grossly abnormal EEG, which developed into a progressive depression terminating in complete silence. Cessation of respiration was followed by anoxic myocardial failure. At death, stimulation of the peripheral end of the cut phrenic nerve elicited a hyperreactive response of the diaphragm, indicating that, with anthrax intoxication, there is no block of the neuromuscular transmission such as has been observed with snake venoms and botulinum toxin. It appeared, rather, that the brain was depressed and no longer capable of initiating an electrical discharge. Spore-infected monkeys displayed a similar EEG pattern (Fig. 8). In both toxin- and spore-challenged monkeys the EKG, blood pressure, and respiratory rate did not deviate from the normal range until shortly before death. In all cases the heart continued to beat for several minutes after respiration ceased.

Antiserum administered to monkeys within 8 hours after challenge with toxin reversed all abnormal physiological changes and all animals survived. However, if administered later than 8 hours, or at one-third of the time required for death of the untreated controls, there was no reversal and the animals died with an extended time to death.

Of the three components of toxin, PA was determined to have caused the initial EEG changes. When purified PA was injected intravenously, immediate changes in EEG and other parameters were observed similar to those following administration of whole toxin; however, all animals rapidly recovered. Purified LF alone did not cause changes in the parameters we measured; all animals survived. When PA was injected, followed in 30 minutes with LF, or vice versa, the same physiological changes were observed as were seen with whole toxin. Specific control for this system was shown when one lot of purified LF and PA that had lost its activity upon lyophilization produced no demonstrable changes. The possibility was thereby ruled out that protein or salts contained in the preparations were causing the observed changes. Toxin inactivated with antisera produced no changes in the physiological parameters monitored.

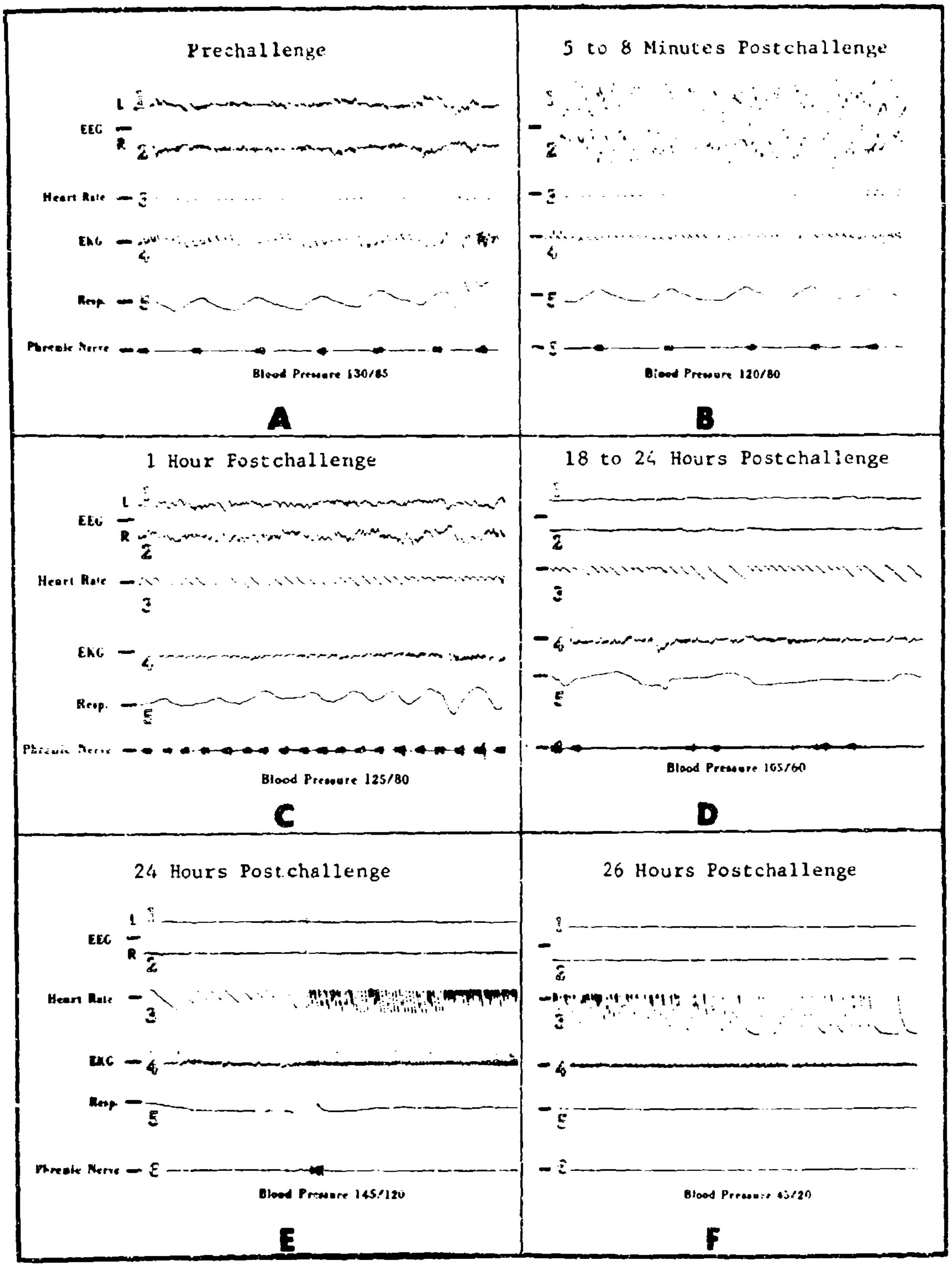

Figure 7. Effect of Anthrax Toxin on EEG, Heart Rate, EKG, Respiration, and Phrenic Nerve Discharges in the Anesthetized Monkey.

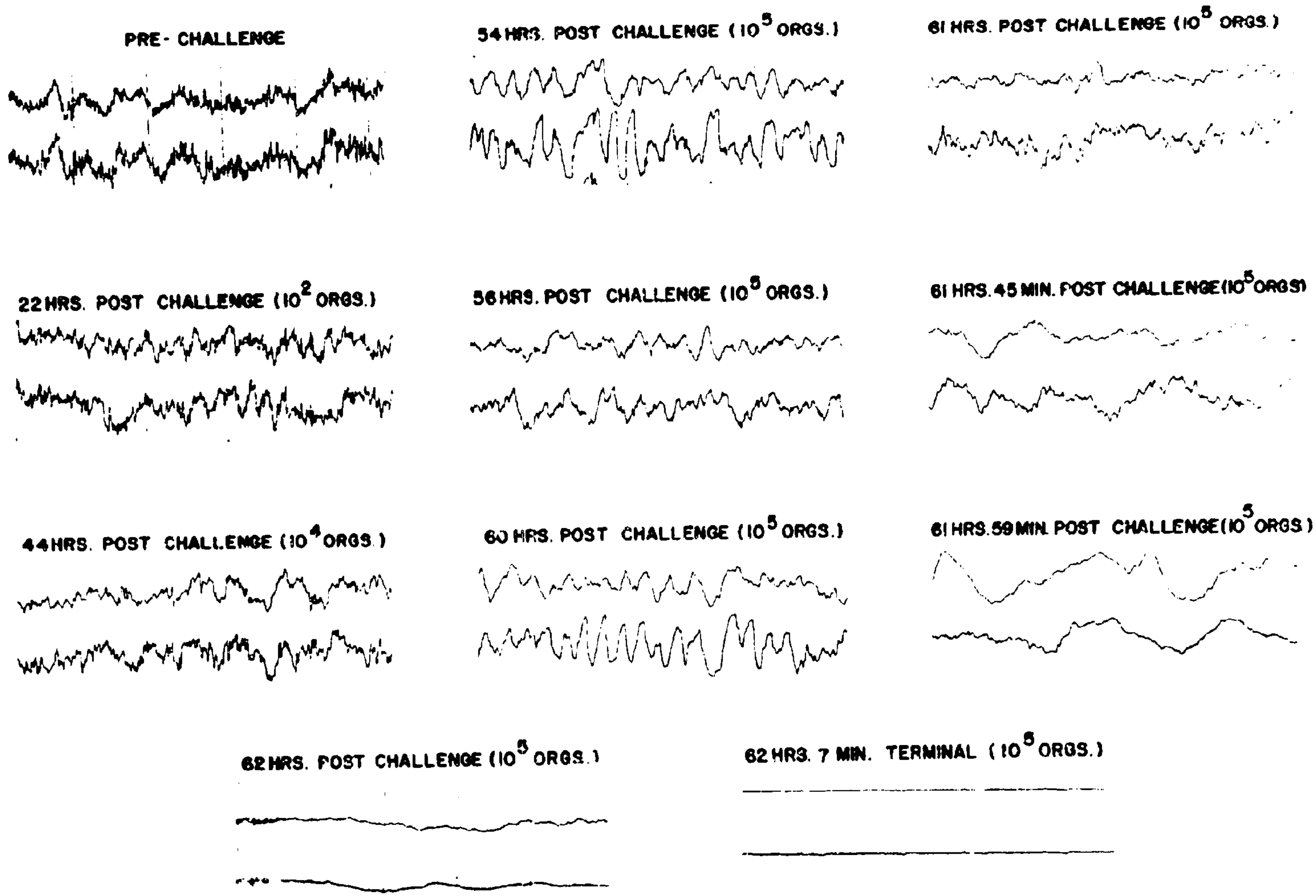

Figure 8. EEG Tracings of the Monkey Pre-Challenge and During Lethal Anthrax Infection.

When as little as 700 units of toxin were injected into the cerebrospinal fluid via the cisterna magna, monkeys died in 6 to 10 minutes and a massive pulmonary edema was observed in contrast to its absence following intravenous toxin administration or following anthrax infection. In all deaths from toxin the heart continued to beat after respiration ceased. Flow rates and blood pressure from the left side of the heart fell sharply and remained low following toxin administration (Fig. 9). Necropsy findings substantiated that the right side of the heart, pulmonary artery, and vena cava were engorged and distended, but the left side was virtually empty.

Our model depicting the disease syndrome, published in 1964,[5] thus has withstood the test of experimentation. This model was constructed on the basis of reports in the literature on sudden death in all species, including man. Disregarding cutaneous lesions, the individual exhibits few symptoms or signs until just before death. Also, our observations, including hyperactivity and spastic paralysis in numerous species of animals dying of anthrax, tended to support this observation. Most changes reported in the literature and our own findings can be explained primarily as CNS involvement. The cellular, biochemical, and physiological alterations appear to be secondary or nonspecific and to contribute to an unknown degree in the death of the host.

In conclusion we can make several points:

There is no doubt that the anthrax disease syndrome is caused by the toxin and not by the organism per se. This knowledge followed the classical work of the Porton group in identifying the toxin. The results of that study indicate that one might reasonably consider anthrax an etiologically new disease, with many similarities to diphtheria and tetanus.

From our observations on several species of animals we conclude that death is caused by toxin acting on the CNS, with depression and paralysis of the respiratory center. Several other parameters also are affected, and at death extreme anoxia, hypoglycemia, and acidosis develop. A decreased Ca^{++} and an increased K^{+} and Cl^{-} were observed. WBC counts and hematocrit values increased; cardiac changes occurred just before respiration ceased.

The pathophysiological changes occurring during either the infectious disease or the toxemic disease are remarkably similar, although an occasional difference, for example, pulmonary edema in the toxemic disease, is noted among the several species of animals with which we worked.

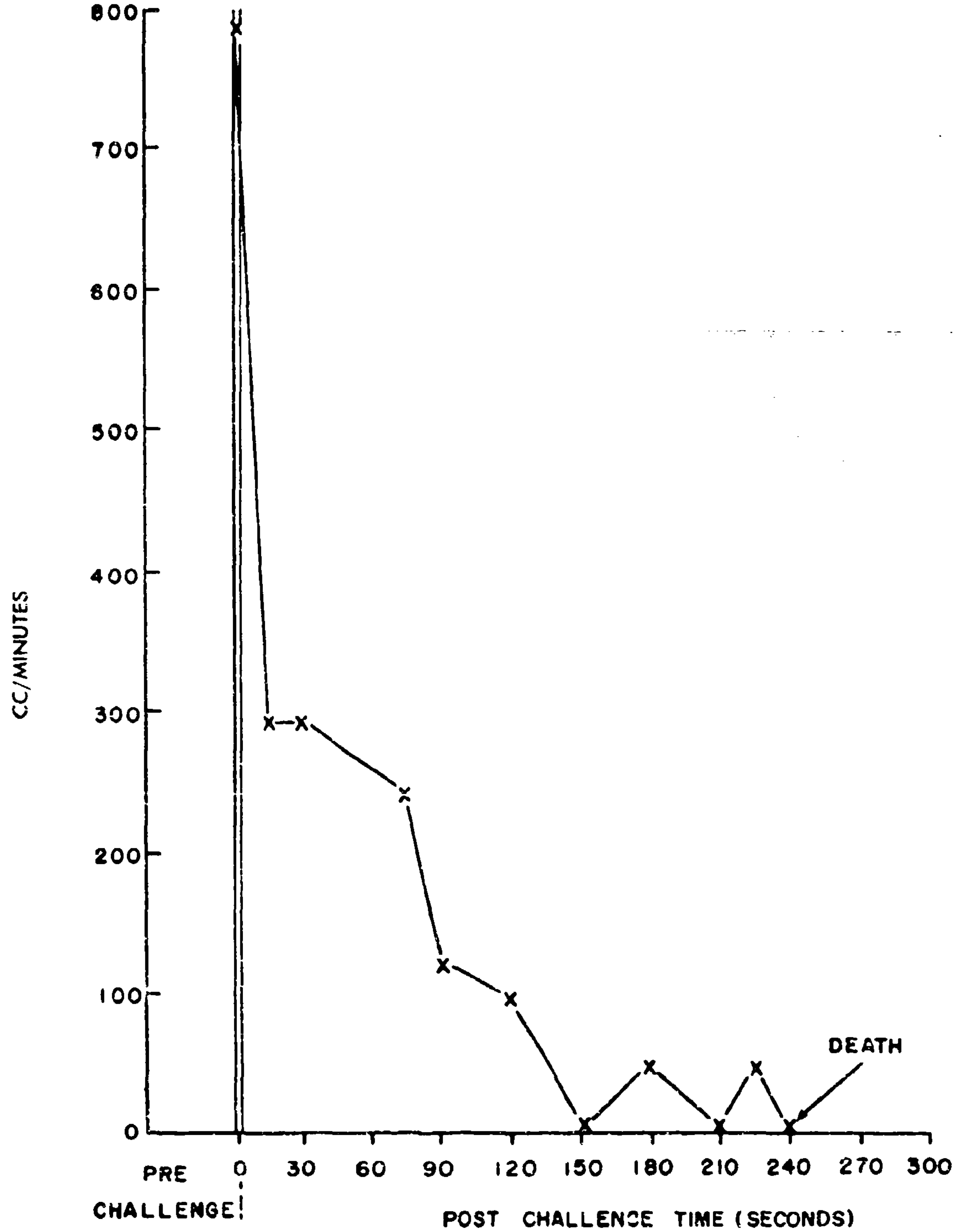

Figure 9. Aortic Flow Rate of the Rhesus Monkey after Challenge with 1,000 Units of *B. anthracis* Toxin by Subdural Injection into Cerebrospinal Fluid.

LITERATURE CITED

1. Smith, H.; Keppie, J.; Stanley, J.L. 1955. The chemical basis of virulence of Bacillus anthracis: V. The specific toxin produced by B. anthracis in vivo. Brit. J. Exp. Pathol. 36:460-472.

2. Toussaint, H. 1877. Sur les bacterides des charbonneuses. Compt. Rend. 85:415.

3. Albrink, W.S. 1961. Pathogenesis of inhalation anthrax. Bacteriol. Rev. 25:268-273.

4. Beall, F.; Dalldorf, F.G. 1966. The pathogenesis of the lethal effect of anthrax toxin in the rat. J. Infect. Dis. 116:377-389.

5. Lincoln, R.E.; Walker, J.S.; Klein, F.; Haines, B.W. 1964. Anthrax. Advances Vet. Sci. 9:327-368.

6. Klein, F.; Walker, J.S.; Fitzpartick, D.F.; Lincoln, R.E.; Mahlandt, B.G.; Jones, W.I., Jr.; Dobbs, J.P.; Hendrix, K.J. 1966. Pathophysiology of anthrax. J. Infect. Dis. 116:123-138.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
PATHOPHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN ANTHRAX

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (Last name, first name, initial)
Walker, Jerry S.
Lincoln, Ralph E.
Klein, Frederick (NMI)

6. REPORT DATE
March 1967

7a. TOTAL NO. OF PAGES
20

7b. NO. OF REFS
6

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 1C522301A059

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
Technical Manuscript 350

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. AVAILABILITY/LIMITATION NOTICES
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Pathophysiological and biochemical changes in anthrax are reviewed. The comparison between spore- and toxin-challenged animals shows a remarkable similarity, indicating that the toxin of Bacillus anthracis caused death although the organisms per se caused little pathophysiological response. Death was primarily due to a respiratory depression of central nervous system origin; the cardiovascular system remained intact. Death occurred with an extreme anoxia that was accompanied late in the disease by numerous secondary or nonspecific changes in the blood cellular, chemical, and gaseous elements.

14. Key Words

*Anthrax	Electroencephalography
*Physiology	Central nervous system
*Blood	Cardiovascular system
*Blood cells	Hypoxia
Toxins	Rhesus monkeys
Spores	Chimpanzees
Biochemistry	Heart
Organisms	Edema
Pathology	Lungs
Respiration	Mortality

DD FORM 1 JAN 64 1473

Unclassified
Security Classification